

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
17 February 2005 (17.02.2005)

PCT

(10) International Publication Number  
**WO 2005/014848 A2**

(51) International Patent Classification<sup>7</sup>:

**C12Q**

(74) Agent: TESKIN, Robin, L.; Crowell & Moring LLP, P.O.  
Box 14300, Washington, DC 20044-4300 (US).

(21) International Application Number:

PCT/US2004/021853

(81) Designated States (unless otherwise indicated, for every  
kind of national protection available): AE, AG, AL, AM,  
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,  
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,  
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,  
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,  
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,  
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,  
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,  
ZW.

(22) International Filing Date: 9 July 2004 (09.07.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/485,745

10 July 2003 (10.07.2003) US

(71) Applicant (for all designated States except US): SENO-  
MYX, INC. [US/US]; 11099 N. Torrey Pines Road, La  
Jolla, CA 92037 (US).

(84) Designated States (unless otherwise indicated, for every  
kind of regional protection available): ARIPO (BW, GH,  
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,  
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,  
FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI,  
SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,  
GW, ML, MR, NE, SN, TD, TG).

(72) Inventors; and

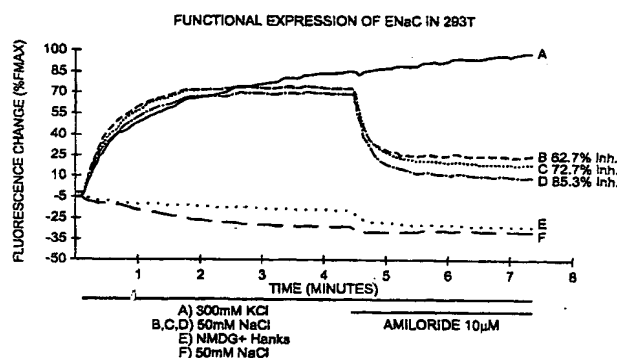
(75) Inventors/Applicants (for US only): SERVANT, Guy  
[CA/US]; 5225 Fiore Terrace, #201, San Diego, CA 92122  
(US). CHANG, Hong [US/US]; 12647 CMTD. DESTUO,  
San Diego, CA 92130 (US). REDCROW, Cyril [CA/US];  
4089 Nobel Drive, #2, San Diego, CA 92122 (US). RAY,  
Sumita [US/US]; 13354 Benchley Road, San Diego, CA  
92130 (US). CLARK, Imran [US/US]; 2352 Caringa  
Way, #A, Carlsbad, CA 92009 (US).

Published:

— without international search report and to be republished  
upon receipt of that report

[Continued on next page]

(54) Title: IMPROVED ELECTROPHYSIOLOGICAL ASSAYS USING OOCYTES THAT EXPRESS HUMAN ENaC AND THE  
USE OF PHENAMIL TO IMPROVE THE EFFECT OF ENaC ENHANCERS IN ASSAYS USING MEMBRANE POTENTIAL  
REPORTING DYES



(57) Abstract: In one aspect, the present invention relates to a mammalian cell-based high-throughput assay for the profiling and screening of human epithelial sodium channel (hENaC) cloned from a human kidney c-DNA library and is also expressed in other tissues including human taste tissue. The present invention further relates to amphibian oocyte-based medium-throughput electrophysiological assays for identifying human ENaC modulators, preferably ENaC enhancers. Compounds that modulate ENaC function in a cell-based ENaC assay are expected to affect salty taste in humans. The assays described herein have advantages over existing cellular expression systems. In the case of mammalian cells, such assays can be run in standard 96 or 384 well culture plates in high-throughput mode with enhanced assay results being achieved by the use of a compound that inhibits ENaC function, preferably an amiloride derivative such as Phenamil. In the case of the inventive oocyte electrophysiological assays (two-electrode voltage-clamp technique), these assays facilitate the identification of compounds which specifically modulate human ENaC. The assays of the invention provide a robust screen useful to detect compounds that facilitate (enhance) or inhibit hENaC function. Compounds that enhance or block human ENaC channel activity should thereby modulate salty taste in humans.



---

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*